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PEER-REVIEWED ARTICLE

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Determining Critical Food Safety Factors for Safely Homebrewing Kombucha: A Study on Microbial Survivability

ABSTRACT

With the surge in popularity of kombucha tea, there has been a growing trend of individuals brewing this beverage at home. However, no consumer recipes have been evaluated by food safety Extension specialists for safety and quality. The present study aimed to determine critical food safety factors necessary for safely homebrewing kombucha. Kombucha was prepared with commercial Symbiotic Culture of Bacteria and Yeasts (SCOBY) and sugar concentrations of 26 g/L, 53 g/L, and 80 g/L prior to inoculation with surrogate organisms *Escherichia coli* K12, avirulent *Salmonella* strain (*Salmonella* Typhimurium strain LT2, H2S+), or *Listeria innocua* to yield nine treatment conditions per replicate, for a total of three replicates. Surrogate populations, titratable acidity of acetic acid (TA), and pH were monitored on Day 0, 7, and 14 of fermentation. TA increased ($p<0.001$) and pH decreased ($p<0.001$) from Day 0 to 14 for all treatments. The total mean log reductions across time period and sugar concentration observed for *E. coli, Salmonella,* and *Listeria* populations were 5.02, 5.86, and 4.26 log CFU/mL, respectively. These findings will be used to inform a validated consumer recipe and corresponding guidance for safely brewing kombucha at home.

INTRODUCTION

Kombucha is a fermented tea beverage, typically brewed from black or green tea *(28)*, with a taste profile comprising both sweet and tart notes, alongside modest effervescence, depending on the brewing approach *(14, 53)*. Historically a beverage of the Far East and Eastern Europe *(27)*, kombucha has gained popular interest by consumers in the United States (U.S.) primarily due to its purported health benefits, which are attributed to its metabolite, probiotic, and micronutrient constituents *(53)*. *In vivo* literature indicates that consistent kombucha consumption may confer some physiological benefit in various animal models of metabolic dysregulation *(2, 15, 24, 35, 39, 44)*. However, the translational potential of these pre-clinical findings to human health outcomes remains to be demonstrated *(15, 40)*.

Critical considerations include the inherent heterogeneity of kombucha formulations in terms of parameters such as car-

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bohydrate source and concentration, contamination events with spoilage microorganisms, microflora composition of the inoculum, tea type, fermentation duration, and fermentation conditions. These factors each have the potential to not only influence health outcomes from consuming the beverage but also the final brew in terms of food safety *(10)*. Ultimately, though popular interest in the potential health benefits from consuming the beverage is increasing, literature substantiating such benefits remains limited in scope and rigor, as comprehensively described in a recent systematic review by Kapp & Sumner *(30)*. Nonetheless, consumers have taken a keen interest in kombucha, thus spurring increased commercial production of the beverage *(31)* and increased consumer interest in making it at home.

Kombucha is made through the fermentation of sweetened tea by a consortium of microorganisms, the Symbiotic Culture of Bacteria and Yeasts (SCOBY), which is scaffolded by a biopolymeric matrix of cellulose to form a biofilm atop the liquid surface of the tea *(55)*. The flavor and safety profiles of the beverage rely primarily on the metabolites of lactic acid bacteria (LAB), acetic acid bacteria (AAB), and yeasts in the SCOBY that are generated over the brewing process, including numerous organic acids *(56, 60)*. Of these, acetic acid is the predominant organic acid in kombucha tea and is understood to greatly contribute to its inhospitable nature toward pathogen survival *(20, 40, 55)* by encouraging a typical pH of 2.5 or greater, but less than 4.2, which is understood to be the critical limit for discouraging growth of bacterial pathogens *(41)*. Kombucha also contains components with demonstrated antimicrobial activity, including polyphenols, lactic acid, and indigenous bacteria, that further impede pathogen survival *(5, 8, 9, 11, 29, 38, 42, 51, 53, 54, 58)*. These components have been recognized for their contribution to the antimicrobial characteristics of kombucha, but the exact mechanisms and pathways remain a subject of debate.

When properly brewed and stored, the beverage is relatively inhospitable to pathogen and mold growth and may even have the capacity to degrade mycotoxins *(7)*. However, when appropriate hygiene and sanitation measures are not taken and/or when key elements of the fermentation, such as pH, are brought outside of generally safe bounds by the consumer, conditions may become welcoming to growth of molds and pathogens introduced through contamination, raising plausible food safety concerns for home brewers not following recipes that have been validated for safety.

To the authors' knowledge, there has not been a confirmed outbreak of illness caused by a foodborne pathogen related to kombucha consumption to date in the United States (U.S.). However, there have been case reports of patients experiencing pathophysiological states, including lead poisoning and lactic acidosis, wherein inappropriate kombucha consumption or preparation approaches, and/or unconfirmed contamination with foodborne pathogens had a suspected role in the respective etiologies *(10, 12, 19, 22, 40, 45, 49, 52, 59)*.

Multiple incidences of lead poisoning in individuals consuming kombucha brewed and/or stored in ceramic containers have been reported *(9, 11, 42)*, wherein the acidity of the beverage leached the mineral from the pot *(40, 45, 59)* to the point that consumers were exposed to harmful levels. Considering these aforementioned cases, it is evident that consumer guidance regarding kombucha handling and preparation can be made more robust and can be more effectively communicated to consumers by researchers in order to better safeguard public health.

The increased demand for validated home food preservation recipes, coupled with the scarcity of available data, highlights the importance of two critical steps: validating recipes to ensure safety of the end-product and effectively communicating these validated recipes to consumers. This is essential not only for preventing foodborne illness but also for mitigating other adverse health consequences that may arise from potentially unsafe home fermentation practices. The research study described herein aimed to establish safety parameters for home-based kombucha brewing. These parameters were determined with a focus on surrogate organism survival, which was assessed in relation to sugar concentration and fermentation duration. Furthermore, this study sought to elucidate the pertinent factors influencing these safety thresholds, such as acidity level, pH, and alcohol content.

MATERIALS AND METHODS

In collaboration with researchers and students enrolled in food microbiology at the University of Georgia (UGA) and Kansas State University (KSU), a challenge study integrating Cooperative Extension and food microbiology teaching lab settings was supported by the National Center for Home Food Preservation (NCHFP) in the fall semester of 2022.

Surrogate strains: Surrogate organisms appropriate for handling at Biosafety Level 1 (BSL-1) were utilized in this study to minimize hazard exposure to the students conducting the research. Selected surrogates were intended to represent the survival behavior of the most probable foodborne pathogens of concern in kombucha products, such as those demonstrated to survive in acidic food milieu and at refrigeration temperatures *(10)*. The strains utilized in this challenge study included *Escherichia coli* K12 (Migula) Castellani and Chalmers ATCC 25404 (*E. coli*), an avirulent *Salmonella enterica* subspecies *enterica* Typhimurium strain LT2, H2S+ (*Salmonella*) (strain provided by Dr. Teresa Bergholz from Michigan State University, Department of Food Science and Human Nutrition), and *Listeria innocua* Seeliger ATCC 33090 (*Listeria*), which served to represent the survival of *E. coli* O157:H7, *Salmonella enterica* subspecies *enterica* serovars, and *Listeria monocytogenes* respectively *(23, 43)*.

Surrogate inocula preparation: Surrogate strains were incubated for 24 h at 35°C in Tryptic Soy Broth (TSB) (Becton, Dickinson & Company, Sparks, MD), with two successive transfers in 24 h intervals. Thereafter, 500 µL of each final inoculum solution was plated onto Tryptic Soy Agar (TSA) (Becton, Dickinson, & Company, Sparks, MD). TSA plates were then incubated for 24 h at 35°C to form a confluent lawn before 5 mL buffered peptone water (Becton, Dickinson, & Company, Sparks, MD) was poured onto plates and a cell scraper used to displace cells. The resulting fluid was transferred to a 15 mL centrifuge tube for each organism, from which 5 mL of inoculum, estimated to have a cell density of approximately 10 log colony forming units (CFU) per mL, was obtained as an inoculum.

Kombucha preparation: Kombucha recipes were prepared in 3.785 L batches, with each batch corresponding to a sugar concentration of 26, 53, or 80 g granulated sugar per liter tea. This specific batch volume and these particular sugar concentrations were selected for this study as they correspond to volumetric quantities of kombucha tea typically made, and sugar amounts typically utilized, in consumer recipes found online for homebrewing kombucha. Tea samples were first brewed for five minutes with 6 sachets of black tea (16 g) and 13 sachets of green tea (34 g) in water preheated to 71–77°C. Brewed tea was allowed to cool to below 35°C prior to inoculation through the addition of a commercial kombucha SCOBY with its starter liquid (SCOBY Kombucha, Clearwater, FL). Subsequently, 3.785 L batches were individually inoculated with surrogate organisms, each at an initial population of 10^6–7 log CFU/mL kombucha solution. Each surrogate (*E. coli*, *Salmonella*, and *Listeria*) was inoculated into a single 3.785 L batch of tea solution per sugar concentration level, resulting in a total of nine treatment conditions per experimental replicate. A total of three experimental replicates were performed. Solutions, covered with paper towels to allow for atmospheric conditions whilset preventing contamination from the environment, were allowed to ferment at ambient temperature (19–23°C), for a total of 14 days, during which time planned sampling events occurred.

Kombucha sampling approach: Prior to each sampling event, each of the nine solutions was stirred with a sterile utensil to homogenize all content. Subsequently, three 10 mL samples and one 20 mL sample were collected from each batch. Ten mL samples were serially diluted in 0.1% buffer peptone water (Fischer Scientific, Pittsburgh, PA) and the pH of the samples was measured post-dilution to confirm neutrality (7.2 ± 0.1 regardless of sugar concentration) prior to spread plating *(29)* on media appropriate for monitoring survival of each surrogate organism. The 20 mL sample was used for monitoring pH and titratable acidity.

Surrogate survival monitoring: Surrogate populations at each sampling point were determined in duplicate on the following agars: MacConkey (MAC) for *E. coli* (Becton, Dickinson, & Company, Sparks, MD), Xylose Lysine Deoxycholate (XLD) for *Salmonella* (Becton, Dickinson, & Company, Sparks, MD), and Oxford Medium Base with Modified Oxford Antimicrobic Supplement (MOX) for *Listeria* (Becton, Dickinson, & Company, Sparks, MD). Sampling events took place at 0, 7, and 14 d after inoculation (hereafter referred to as days), at which time plating occurred. All media were incubated for 48 h at 35°C prior to enumeration.

Antimicrobial activity: An agar well diffusion assay with overlay was performed to evaluate the potential antimicrobial activity of bacteriocins or other uncategorized antimicrobials from the kombucha sample that may have impaired recovery of target organisms. TSA plates were prepared and 7 mm wells were created with sterile pipettes. In each TSA plate, a total of five wells were created. Ten µL of kombucha samples were pipetted into each well. In each plate, three wells were allocated for kombucha and two wells for negative controls filled with sterile deionized water. One mL of 24 h culture of each surrogate was inoculated in 5 mL molten agar tempered to 50 °C. The inoculum was then poured atop the plate, such that all wells were covered by the inoculum. The plates were then incubated at 37°C for 24 ± 2 h for *E. coli* and *Salmonella*, and 48 ± 2 h for *Listeria*. The antimicrobial activity was evaluated by measuring the zone of inhibition surrounding the wells. Each kombucha sugar concentration was carried out in triplicate biological replicates.

pH monitoring: pH of the kombucha solution was monitored across the 14-day fermentation period at each sampling event in the challenge study using the 20 mL kombucha sample and a calibrated pH meter (accumet AE150, Fisher Scientific, Waltham, MA).

pH of SCOBY, SCOBY starter liquid, and tea: pH of the SCOBY, starter liquid, and tea prior to inoculation with SCOBY was measured subsequent to the challenge study using a portable multimeter (Hach HQ4200). The kombucha sampled was prepared with 53 g/L granulated sugar and was not inoculated with surrogate organisms, otherwise preparation of the kombucha was identical to the methodology described above.

Titratable acidity of acetic acid (TA) determination: TA was also monitored on Day 0, 7, and 14 of fermentation using acid-base titration with 0.1 N or 1.0 N NaOH, as appropriate, with an endpoint pH of 8.3. Initially, 0.1 N NaOH was utilized due to the relatively low level of acid in the kombucha. Into the later stages of the challenge study, 1.0 N NaOH was utilized in some cases due to the increased TA. TA was calculated using the following equation per the concentration of alkali utilized at each sampling point, where Z = 0.1 N or 1.0 N NaOH:

$$TA = \frac{Z\,\frac{mEq}{mL}\,NaOH \times x\,mL\,NaOH \times 60.06\,\frac{mg}{mEq}}{20\,mL \times 10\,\frac{mg}{mL}}$$

TABLE 1. Analysis of variance of log reduction for each organism explained by time period, sugar concentration, and their interaction

Source of Variation	*E. coli*	*Listeria*	*Salmonella*
	P-value		
Time period	<0.001	<0.001	<0.001
Sugar concentration	<0.001	<0.001	0.057
Time period x sugar concentration	<0.001	0.138	<0.001

The factor for acetic acid used in these calculations was that from the Association of Official Agricultural Chemists International (AOAC) Official Method℠ 942.15 *(25)*.

Ethanol content: Ethanol content of kombucha was monitored subsequently to the challenge study in accordance with AOAC Official Method℠ 2017.07, as described by Lacorn and colleagues *(34)*, except for the degassing step, which was omitted due to samples not containing carbonation. Ethanol level was measured on Day 0, Day 7 through 10, and on Day 14 of fermentation and calculated as percent alcohol by volume (%ABV). A 340 nm wave spectrophotometer (Beckman DU530 UV-Vis) was used to quantify analyte.

Statistical analyses: Log reduction was analyzed separately for each of the three surrogate organisms with a mixed-effect analysis of variance model where period (0 to 7 days, 7 to 14 days), sugar concentration, and their interaction were treated as fixed effects, and block was treated as random effects. These models were also run with a repeated measure error structure (i.e., compound symmetry), and the model presenting the best fit based on lowest Akaike Information Criteria (AIC) was selected for further inference. The pH and total acidity analysis of variance were each conducted using a mixed-effect model where days of incubation *(0, 7, 14)*, sugar concentration, and their interaction were treated as fixed effects, and block was treated as random effect. For all models, significant effects were further analyzed by performing Fisher's LSD pairwise comparisons at alpha = 0.05. All data processing, analysis, and graphing were conducted in R version 4.3.1 *(47)*. Mixed-effect ANOVA models were conducted using function lmer from package lme4 *(4)*.

RESULTS AND DISCUSSION

Surrogate mean log reductions: *E. coli* and *Salmonella* mean log reductions were significantly affected by the interaction of time period and sugar concentration, illustrated in *Table 1*.

Mean log reduction in *E. coli* from 0 to 7 days was greatest at sugar concentrations of 26 and 53 g/L (4.77 and 5.02 log CFU/mL, respectively), and lowest at 80 g/L (3.11 log CFU/mL). Mean log reductions from 7 to 14 days were statistically similar across the different sugar concentrations and varied from 0.35 to 0.46 log CFU/mL. Mean log reductions in *E. coli* populations from 7 to 14 days were significantly lower than those from 0 to 7 days ($p<0.05$).

Similar trends were observed for *Salmonella*, where mean log reduction from 0 to 7 days was greatest at sugar concentrations of 26 and 53 g/L (5.86 and 5.57 log CFU/mL, respectively), and lowest at 80 g/L (4.00 log CFU/mL). Mean log reductions from 7 to 14 days were statistically similar and varied from 0.35 to 0.50 log CFU/mL across the different sugar concentrations. Mean log reductions in *Salmonella* from 7 to 14 days were significantly lower than those from 0 to 7 days ($p<0.05$). Findings from a previous challenge study evaluating the survival of three *Salmonella* serotypes and Shiga toxin-producing *E. coli* (STEC) in home-brewed kombucha made from various commercial brewing kits demonstrated similar trends in population log reduction *(10)*. *E. coli* and *Salmonella* population trends across the 14-day fermentation process are illustrated in *Figure 1*.

Listeria mean log reductions were significantly affected by time period ($p<0.001$) and sugar concentration ($p<0.001$). A total log reduction of 2.38 and 2.44 log CFU/mL was observed at sugar concentrations of 26 g/L and 53 g/L, respectively. The lowest reduction (1.80 log CFU/mL) was observed at 80 g/L, which was significantly lower than those at 26 g/L and 53 g/L, as shown in *Figure 2*.

Figure 3 depicts the significant difference in mean log reductions of *Listeria* between time periods. The reduction observed from 0 to 7 days (4.26 log CFU/mL) was greater ($p<0.05$) relative to that observed from 7 to 14 days (0.36 log CFU/mL). Using the agar well diffusion method, Al-Mohammadi and colleagues have demonstrated that kombucha may have antimicrobial activity against *Listeria monocytogenes (1)*, and Diguță and colleagues demonstrated that certain strains of *Pediococcus* spp. sourced from commercial kombucha inhibited *Listeria monocytogenes (18)*.

These findings indicate that 26 g/L and 53 g/L sugar concentration levels support a greater total mean log reduction in *E. coli*, *Salmonella*, and *Listeria* in kombucha relative to 80 g/L from Day 0 to Day 14 of fermentation. This outcome is hypothesized to be attributed to decreased competition for resources among the microflora in kombucha solutions with 80 g/L of carbohydrate source

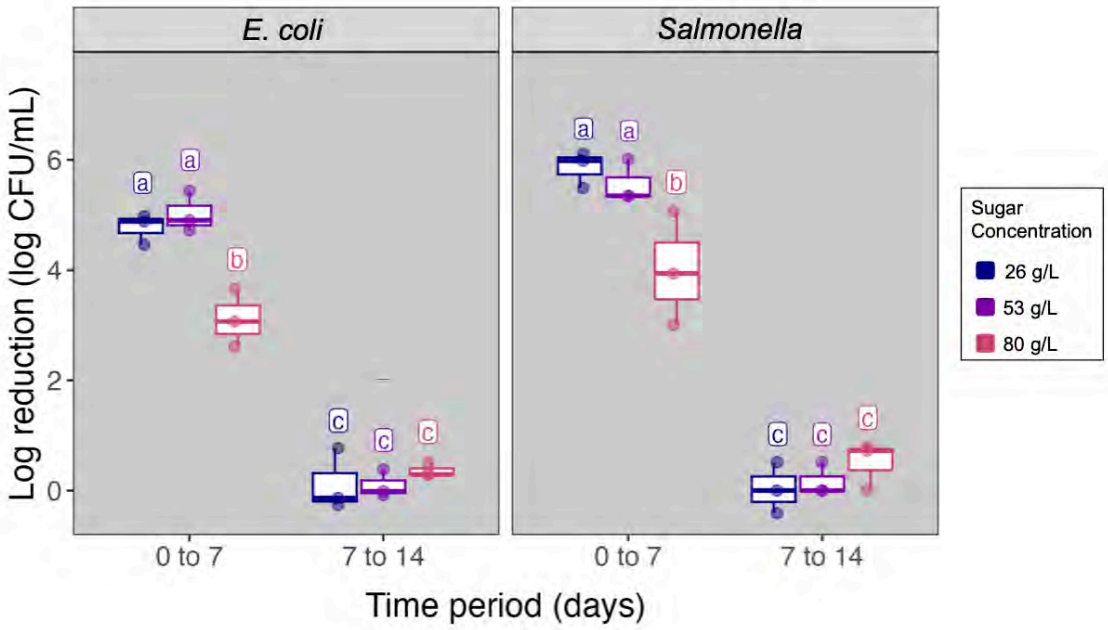

Figure 1. Boxplot of *E. coli* and *Salmonella* mean log reductions (log CFU/mL) at different time periods and sugar concentrations. Boxplots preceded by the same letter within an organism are not significantly different at alpha = 0.05.

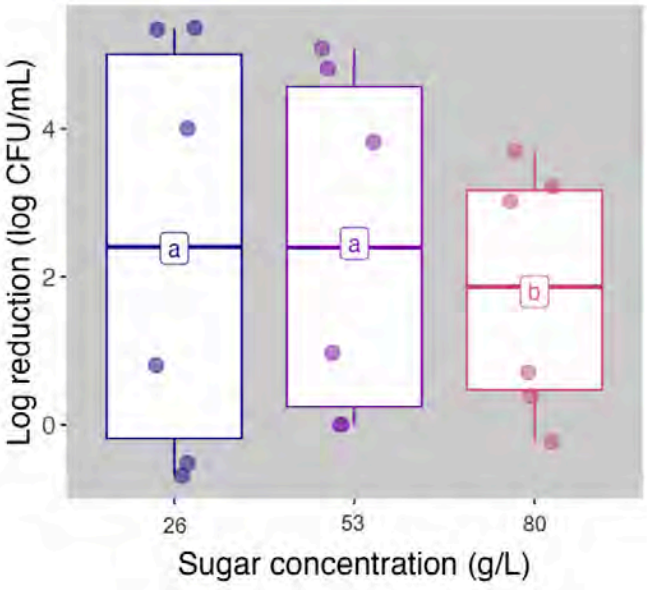

Figure 2. Boxplot of *Listeria* total mean log reductions (log CFU/mL) at different sugar concentrations. Boxplots with the same letter are not significantly different at alpha = 0.05.

relative to solutions with lower sugar concentrations. With more carbohydrate substrate in 80 g/L solution for the microflora present at the onset of fermentation, the degree to which nutrient availability in the environment would serve as a limiting factor to survivability of pathogens would be limited *(6)*. This may have allowed for greater survival of surrogate populations in this relatively more nutrient-abundant environment as compared to the kombucha preparations with lower sugar concentrations of 26 g/L and 53 g/L *(21, 46)*.

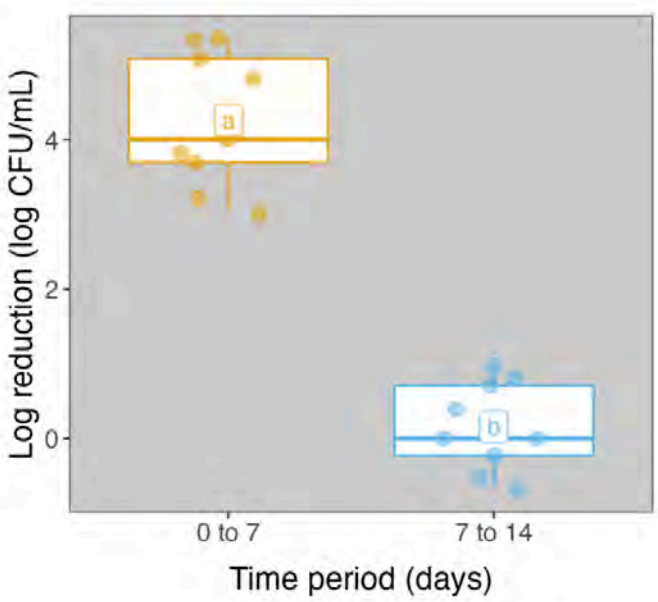

Figure 3. Boxplot of *Listeria* mean log reduction (log CFU/mL) at different time periods. Boxplots with the same letter are not significantly different at alpha = 0.05.

TABLE 2. Analysis of variance in pH explained by day, sugar concentration, and their interaction

Source of Variation	*E. coli*	*Listeria*	*Salmonella*
	P-value		
Day	<0.001	<0.001	<0.001
Sugar concentration	0.005	0.136	0.046
Day x sugar concentration	0.308	0.288	0.163

pH: pH was significantly affected by day and sugar concentration in batches inoculated with *E. coli* and *Salmonella* and only by day for batches inoculated with *Listeria*, as illustrated in *Table 2*. This outcome may be attributed to relatively more acid-tolerant strains of *Listeria* being present in the tested batch relative to *E. coli* or *Salmonella*. Certainly, *Listeria monocytogenes* has demonstrated the capacity to sustain more acidic environments, particularly when first exposed to certain sub-lethal stressors *(13, 32)*.

The average initial pH values of the SCOBY pellicle, starter liquid, and tea prior to inoculation with the SCOBY were 2.78, 2.77, and 5.82, respectively, on Day 0. The initial mean pH of kombucha on Day 0 of fermentation after inoculation with SCOBY was approximately 3.88 across all nine treatment conditions, significantly decreasing to an average of 3.09 across all treatment conditions by Day 14 of fermentation ($p<0.05$). Trends in pH across the three sampling events are illustrated in *Figure 4*.

As previously mentioned, the acidity of kombucha is one of the primary factors making the beverage relatively inhospitable to the survival and growth of bacterial pathogens. However, it is hypothesized that the acidity of the beverage may also be a key contributing factor to the cases of lactic acidosis that have occurred in relation to kombucha consumption *(40)*, and therefore, it is not recommended for the pH of kombucha to drop below 2.5 without some form of corrective action *(41)*. Rather, it is generally recommended that kombucha pH values range between 2.5 and 4.2 in order to be inhospitable to bacterial pathogens but not be acidic enough to potentially be harmful to individuals who may be more vulnerable to developing acidotic health states when regularly consuming kombucha *(16, 40, 57)*.

Sugar concentration had a significant effect on pH decrease in kombucha inoculated with *E. coli* or *Salmonella*. The lowest pH was observed at the highest sugar concentration (80 g/L), 3.45 and 3.40 for *E. coli* and *Salmonella*, respectively.

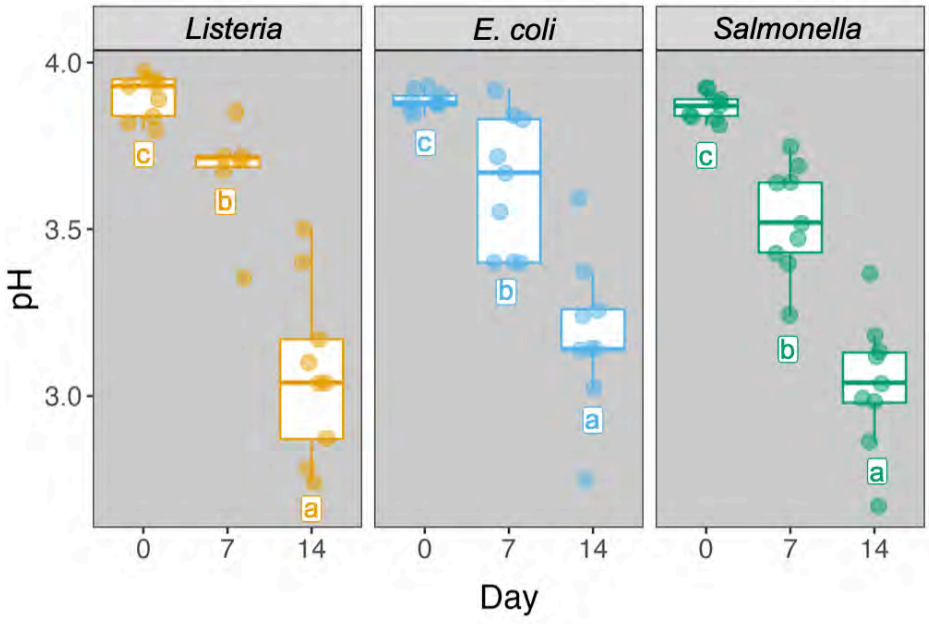

Figure 4. Boxplot of pH readings at each sampling point in kombucha inoculated with *Listeria*, *E. coli* and *Salmonella*. Boxplots followed by the same letter within an organism are not significantly different at alpha = 0.05.

The highest pH was observed at 26 g/L of sugar with values of 3.67 and 3.56, for *E. coli* and *Salmonella*, respectively (shown in *Figure 5*).

Antimicrobial activity: No zone of inhibition was observed in the kombucha bacteriocin assay, indicating a lack of antimicrobial activity under the conditions tested. In contrast, previous studies have identified antimicrobial activity against *Helicobacter pylori*, *Staphylococcus aureus*, *Agrobacterium tumefaciens*, and *Bacillus cereus (20)*. The discrepancies between our findings and those of others may be attributed to factors such as the degradation or inactivation of the bacteriocin during fermentation, or the inherent resistance of the target microorganisms. Future studies should focus on isolating and characterizing specific bacteriocins produced during kombucha fermentation while examining how factors such as fermentation time, temperature, and starter culture composition influence their production and antimicrobial efficacy. Additionally, investigating the interactions between non-pathogenic and pathogenic bacteria in kombucha, along with genomic and proteomic analyses, could enhance our understanding of the role of bacteriocins in fermentation.

Titratable acidity of acetic acid (TA): TA values were only significantly affected by day, as shown in *Table 3*. The pKa of acetic acid, 4.76 *(50)*, relative to the pH of the solution at any one time, determines its antimicrobial capacity *(33)*. Acetic acid molecules in a solution with pH < 4.76 will primarily be in their undissociated form, wherein they retain their antimicrobial capacity by being able to penetrate the cell membrane of microorganisms in solution prior to dissociating in the more neutral environment of the cytoplasm, acidifying the intracellular fluid over time and inactivating cellular machinery, eventually causing cell death *(57-58)*. Indeed, in jalapeño and serrano peppers, Rangel-Vargas and colleagues demonstrated antimicrobial action of acetic acid against some of the foodborne pathogens that strains in the present study served as surrogates for, including *E. coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Montevideo, and *Listeria monocytogenes (48)*.

TA increased over the course of fermentation ($p<0.001$), with a majority of the increase occurring from Day 7 to 14 of the fermentation, as depicted in *Figure 6*. This not only demonstrated an increasing contribution of acetic acid to the acidity of the kombucha solution as the fermentation proceeded, but also an increasing proportion of acetic acid molecules in solution existing in their undissociated form, as opposed to their dissociated form, as pH continued to drop below 4.76. This reflects an increasing proportion of acetic acid conferring antimicrobial capacity to the kombucha as the fermentation progressed.

Mean TA values were 0.11%, 0.15%, and 0.12% for tea inoculated with *E. coli*, *Salmonella*, and *Listeria* respectively, on Day 0 of fermentation. These values increased to 0.16%, 0.19%, and 0.16% by Day 7, and they each increased significantly to 0.47%, 0.62%, and 0.60% by Day 14 of fermentation ($p<0.05$). Wang and colleagues determined the TA for kombucha samples made from commercial starter culture sourced from New Zealand to be within the range of

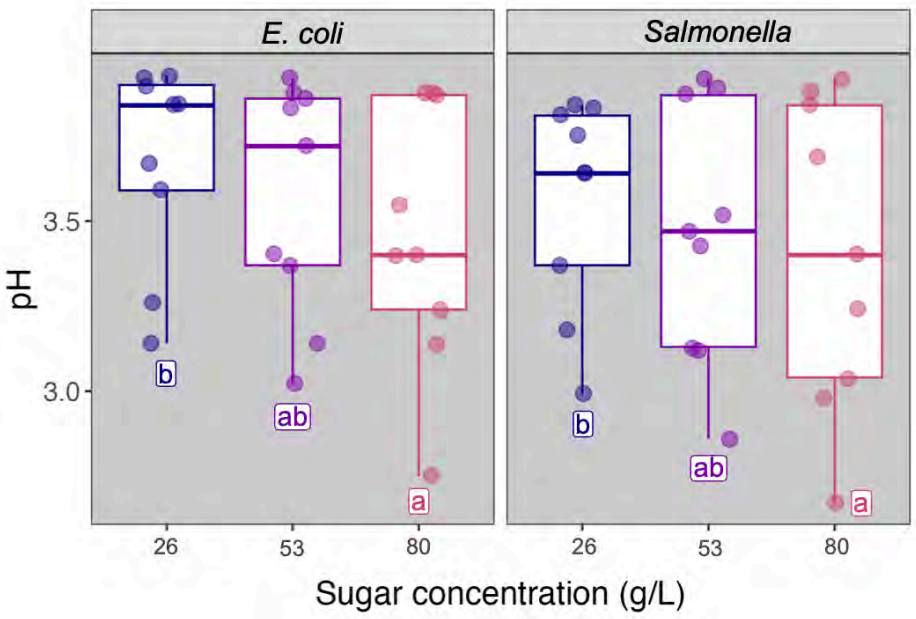

Figure 5. Boxplot of pH at different sugar concentrations for kombucha inoculated with *E. coli* and *Salmonella*. Boxplots followed by the same letter within an organism are not significantly different at alpha = 0.05.

TABLE 3. Analysis of variance of TA explained by day, sugar concentration, and their interaction

Source of Variation	*E coli*	*Listeria*	*Salmonella*
	P-value		
Day	<0.001	<0.001	<0.001
Sugar concentration	0.975	0.819	0.543
Time period x sugar concentration	0.983	0.62	0.228

0.38% to 0.43% *(57)*. Relative differences in TA determined through this study may be attributed to differing microflora composition in the respective commercial starter cultures, as well as differences in ingredients and fermentation methodology used in each fermentation.

Ethanol: As depicted in *Table 4*, initial ethanol content in the kombucha sample on Day 0 of fermentation was approximately 0.016% alcohol by volume (%ABV). This level was maintained across Day 7 through 10 of sampling before rising to 0.018 %ABV by Day 14 of sampling.

In the U.S., the Alcohol and Tobacco Tax and Trade Bureau (TTB) regulates alcoholic beverages destined for commerce, including kombucha if it reaches a %ABV greater than or equal to 0.5% at any point during production. At that point, pursuant to 27 CFR § 25.15, the beverage would be considered a beer if the fermentation in the given product is driven by sugar or some other malt-alternative substrate *(61)*, and otherwise as a wine.

Typically, kombucha sold commercially in the U.S. contains minimal alcohol. However, the British Columbia Centre for Disease Control (BCCDC) conducted a recent study which found that approximately one third of the 684 kombucha samples obtained from 53 domestic and non-domestic processors had %ABV values, as determined by headspace GC-MS, in excess of British Columbia, Canada's regulatory maximum level of 1% *(26)*, and thus well above the regulatory limit set by the TTB for products sold in the U.S.

Furthermore, the authors found that 12.7% of the kombucha samples imported from U.S. producers exceeded 1% ABV *(26)*. Therefore, populations especially vulnerable to the negative consequences of alcohol consumption, such as pregnant individuals, are typically advised to abstain

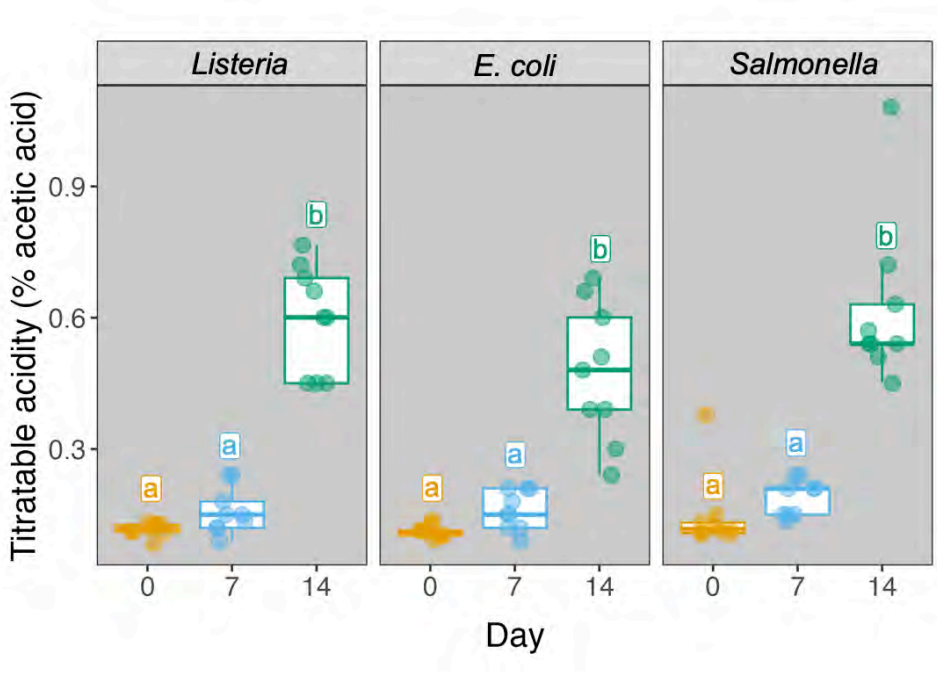

Figure 6. Boxplot of TA at each sampling time point for kombucha inoculated with *Listeria*, *E. coli* and *Salmonella*. Boxplots preceded by the same letter within an organism are not significantly different at alpha = 0.05.

TABLE 4. Average %ABV and standard deviation at each sampling point in kombucha monitored across a 14-day fermentation period

Day of Fermentation	Ethanol Content (%ABV)
0	0.016±0.00022
7	0.016±0.00055
8	0.016±0.0011
9	0.016±0.00032
10	0.016±0.00022
14	0.018±0.00085

from consuming kombucha, in part because ethanol level in kombucha may be uncertain and also because, regardless of the %ABV in the beverage, there remains no known safe level of alcohol one may consume during pregnancy without potentially teratogenic effects to the fetus *(17, 26, 37)*.

Some limitations of this study include limited sampling events and limited treatment variables (such as types of sugar and teas) due to time and resource constraints. Expanding upon this challenge study, future research might explore the effects of using different types of tea and/or carbohydrate sources than those described for this project, survival of surrogates in the setting of a continuous brew, mycotoxin production across the fermentation process, or the influence of flavoring additives in the brew on surrogate survival.

CONCLUSIONS

The findings from this study are being utilized by the NCHFP to inform a validated kombucha recipe to be shared with consumers through Cooperative Extension. Providing a readily accessible validated recipe, alongside the findings from the validation study, to consumers will be instrumental in enhancing the prevention of foodborne disease in consumers who wish to ferment kombucha at home. The continued commitment of researchers to conduct validation studies on home food preservation recipes and communicate the results of these validation studies for public access will contribute to the development of a more comprehensive repository of evidence-based recipes, thereby empowering consumers with safer and more reliable options.

Furthermore, effectively communicating these recipes through Cooperative Extension will bring enhanced visibility of validated recipes to consumers, thus reducing the public health impacts of illness incurred through unsafe home food preservation practices.

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